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(54) Titre : CAMEL, AUTRE PRODUIT DE TRADUCTION DE L'ANTIGENE TUMORAL LAGE-1

(54) Title: CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1

(57) Abrégé/Abstract:

The tumor-associated antigen CAMEL and DNA encoding it. The tumor-associated antigen is encoded by an open reading frame of the LAGE-1 gene. The tumor-associated antigen, immunogenic (poly)peptides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.



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| (54) Title: <b>CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1</b>   |  |  |  |
| <p>CAMEL -----</p> <p>LAGE-1<sup>s</sup> --ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG 48</p> <p>LAGE-1<sup>L</sup> GCATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG 50</p> <p>NY-ESO-1 --ATCCTCGTGGGCCCTGACCTTCTCTCTGAGAGCCGGGCAGAGGCTCCG 48</p> <p>CAMEL -----CGACGGGCGATGCT 14</p> <p>LAGE-1<sup>s</sup> GAGCCATGCAGGCCGAAGGCCAGGGCACAGGGGGTTCGACGGGCGATGCT 98</p> <p>LAGE-1<sup>L</sup> GAGCCATGCAGGCCGAAGGCCAGGGCACAGGGGGTTCGACGGGCGATGCT 100</p> <p>NY-ESO-1 GAGCCATGCAGGCCGAAGGCCAGGGCACAGGGGGTTCGACGGGCGATGCT 98</p> <p>*****</p> <p>CAMEL GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCAGGGGCAATGCTGG 64</p> <p>LAGE-1<sup>s</sup> GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCAGGGGCAATGCTGG 148</p> <p>LAGE-1<sup>L</sup> GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCAGGGGCAATGCTGG 150</p> <p>NY-ESO-1 GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCAGGGGCAATGCTGG 148</p> <p>*****</p> <p>CAMEL CGGCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG 114</p> <p>LAGE-1<sup>s</sup> CGGCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG 198</p> <p>LAGE-1<sup>L</sup> CGGCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG 200</p> <p>LAGE-1<sup>L</sup> CGGCCAGGAGAGGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG 198</p> <p>*****</p> |  |  |  |
| (57) Abstract   |  |  |  |
| <p>The tumor-associated antigen CAMEL and DNA encoding it. The tumor-associated antigen is encoded by an open reading frame of the LAGE-1 gene. The tumor-associated antigen, immunogenic (poly)peptides derived therefrom and DNAs encoding them, are useful for cancer immunotherapy.</p>   |  |  |  |

**CAMEL, AN ALTERNATIVE TRANSLATION PRODUCT OF THE TUMOUR ANTIGEN LAGE-1**

The present invention relates to the field of cancer therapy, more specifically to tumor-associated antigens.

Cytotoxic T lymphocytes (CTLs) play an important role in the defense  
5 against melanoma. Melanoma-specific CTL clones have been obtained  
from either tumor infiltrating lymphocytes (TIL) *in vitro* stimulated with  
cytokines, or from peripheral blood mononuclear cells (PBMC) cultured with  
(autologous) tumor cells. T cell responses against tumor cells are enhanced  
by cytokine transfection of the tumor cells, both in animal models and in *in*  
10 *vitro* human culture systems. (van Elsas et al., 1997; Gansbacher et al.,  
1990; Tepper et al., 1989; Fearon et al., 1990; Dranoff et al., 1993)

The antigens recognized by the tumor-specific T cells become better  
defined by the development of molecular cloning techniques. These T cell  
targets can be divided in three groups: 1) tumor-specific antigens, not  
15 expressed in healthy tissues, except testis and placenta (e.g., MAGE,  
BAGE, GAGE, NY-ESO-1, LAGE-1); 2) antigens that are lineage-specific  
and expressed in both melanoma and melanocytes (e.g., MART-1/ Melan-  
A, gp100, tyrosinase) and 3) unique, mutated antigens (e.g.,  $\beta$ -catenin,  
CDK4, MUM-1) (reviewed by Van den Eynde and Brichard, 1995).

20 By means of Representational Difference Analysis (RDA), a PCR-based  
method that has been used to identify genes with tissue-specific or tumor-  
specific expression, the LAGE-1 and NY-ESO1 genes were identified as  
being tumor specific by screening cDNA libraries from melanoma cell lines  
with a primer from a cDNA clone enriched in melanoma-specific sequences  
25 (Lethe et al., 1998).

NY-ESO-1 is a gene originally identified by SEREX technology (Chen et al.,  
1997). It was shown to have two different reading frames (DNA sequences  
and derived protein sequences given in SEQ ID NO: 7 - 10), translation

products of which were shown to contain epitopes of tumor specific T-cells (Jäger et al., 1998; Wang et al., 1998).

It was an object of the present invention to provide a novel tumor-associated antigen.

- 5 To solve the problem underlying the invention, melanoma cell line 518A2 and its IL-2- or GM-CSF-transfectants were compared for their CTL stimulating capacity *in vitro*. Stimulation of autologous PBMC with the IL-2 producing melanoma cells resulted in a melanoma-specific CTL response (van Elsas et al., 1997). CTL clones derived from this culture recognized,  
10 besides autologous melanoma cell lines, also a panel of HLA-A\*0201 positive melanoma cell lines, but were not reactive with normal melanocytes. Although 518A2 was shown to express a number of antigens previously identified to be recognized by anti-melanoma CTL (van Elsas et al., 1996), the CTL clones available recognize a new melanoma-specific  
15 antigen that is immunodominant in 518A2.

In the experiments of the present invention, the target structure that was recognized by one of these CTL clones was fully characterized and named CAMEL (CTL-recognized Antigen on Melanoma). These sequences are described in the attached sequence listing as SEQ ID NO: 1 and SEQ ID  
20 NO: 2.

It was surprisingly found that CAMEL is encoded by a reading frame of the LAGE-1<sup>s</sup>-cDNA (SEQ ID NO:3) that is distinct from that encoding the putative LAGE-1 protein (SEQ ID NO: 4). (This reading frame is designated ORF-1.)

- 25 In the present invention, a cDNA clone was identified that lacks the first 84 bp of the LAGE-1<sup>s</sup> sequence (SEQ ID NO: 3) which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in this clone (4H8) is the ATG at position 94 of LAGE-1<sup>s</sup> (SEQ ID NO: 3), which is however, not in frame with

the first ATG at position 54. Therefore, the CAMEL protein (SEQ ID NO: 2) translated from the 4H8 cDNA clone is different from the putative LAGE-1<sup>S</sup> protein (SEQ ID NO: 4).

5 In a first aspect, the present invention is directed to the tumor-associated antigen CAMEL (SEQ ID NO: 2) which is encoded by an isolated DNA molecule with the sequence as defined in SEQ ID NO: 1.

The coding sequence of CAMEL corresponds to the ORF-1 of LAGE-1 cDNA (Lethe et al., 1997; WO 98/32855).

10 In the present invention „ORF-1“ is defined as the open reading frame starting with ATG at position 94 of SEQ ID NO:3 (LAGE-1<sup>S</sup>), which corresponds to position 10 in SEQ ID NO: 1 (CAMEL), to position 96 in SEQ ID NO: 5 (LAGE-1<sup>L</sup>)

15 In a further aspect, the present invention relates to immunogenic (poly)peptides derived from CAMEL. A first group of peptides is selected from peptides inducing a humoral immune response (induction of antibodies). Such peptides are selected by randomly choosing continuous stretches of amino acids (at least 12-15), applying them to an individual and confirming the generation of antibodies by standard immunological assays, e.g. ELISA. This group of immunogenic (poly)peptides also encompasses  
20 the entire CAMEL antigen or larger fragments thereof.

The second group of peptides, which is preferred, can be presented by MHC molecules (in humans: HLA molecules), they have the potential to induce an immune response, in particular by eliciting a CTL response.

25 In a preferred embodiment, immunogenic peptides which have the ability to elicit a CTL response, are selected from peptides with the sequence set forth in SEQ ID NO: 11, 12, 24, 25 and 26.

To obtain peptides that have the ability to elicit a cellular immune response, the selection of peptide sequences from a given antigen is, in the first place, based on the requirement for such peptide to bind to an MHC molecule present in the repertoire of the patient to be treated. Two classes of MHC  
5 molecules are distinguished, class I and class II. Class I molecules consist of a membrane-inserted heavy chain and a non-covalently attached light chain. In their structure, MHC class I molecules resemble a moose's head, the antlers forming a groove which is recognized by the peptide. In humans, HLA-A, B and C are the "classical" MHC class I molecules.

10 Additional immunogenic peptides may be identified by methods known in the art which rely on the correlation between MHC-binding and CTL induction, e.g. those used by Stauss et al., 1992, who identified candidate T-cell epitopes in human papilloma virus.

15 Since immunogenic peptides can be predicted based on their "peptide binding motif" synthetic peptides which represent CTL epitopes may be designed and synthesized. Several methods, which are useful in the present invention for designing peptides, have been used to identify CTL epitopes from known protein antigens.

It is well established that every MHC class I allelic product has  
20 allele-specific requirements for the peptide ligand that binds to its groove and that it ultimately presents. These requirements were summarized as a motif by Falk et al., 1991. A large number of MHC peptide motifs and MHC ligands have become known to date. A method to search a known protein sequence for epitopes fitting to a given class I molecule, which is  
25 based on this knowledge and which may be used in the present invention, was reviewed by Rammensee et al., 1995. It comprises the following steps: first, the protein sequence is screened for stretches fitting to the basic anchor motif (two anchors in most cases), whereby allowance should be made for some variation in peptide lengths as well as in anchor occupancy.  
30 If a motif, for example calls for 9mers with Ile or Leu at the end, 10mers with

a fitting C-terminus should be considered as well, and other aliphatic residues at the C-terminus, like Val or Met, should also be considered. In this way, a list of peptide candidates is obtained. These are inspected for having as many non-anchor residues as possible in common with ligand already known, or with the residues listed among the "preferred residues" or "others" on top of each motif (Table, given by Rammensee et al., 1995), for various HLA molecules. Binding assays can be performed at this stage to exclude weak binders which occur frequently among peptides conforming to a basic motif. If a detailed study on peptide binding requirements is available, the candidates can also be screened for non-anchor residues detrimental or optimal for binding (Ruppert et al., 1993). One should keep in mind, however, that pure peptide binding motifs can be misleading in the search for natural ligands, since other constraints, such as enzyme specificity during antigen processing and specificity of transporters or chaperones, are likely to contribute to ligand identity in addition to the MHC binding specificity.

This approach was successfully applied by, inter alia, Kawakami et al., 1995, to identify gp100 epitopes based on known HLA-A2.1 motifs. The validity of the method was confirmed by identifying, in parallel, the epitope regions by using COS cells transfected with cDNA fragments generated by sequential deletion and testing for T-cell reactivity, as described above.

Recently, data bases and prediction algorithms have become available that enable to predict, with great reliability, peptide epitopes that bind to HLA molecules of interest.

Examples for peptide candidates with potential immunogenicity that can be derived from the tumor-associated antigen of the present invention, are the CAMEL-derived peptides with the sequence HLSPDQGRE and LMAQEALAF for HLA-A3 or RMAVPLLRR for HLA-A3101. Similarly, other peptides for these or for other alleles can be determined by the method mentioned above.

The peptide binding can be tested in peptide binding assays. In order to determine the immunogenicity of the selected peptide or peptide equivalent, as defined below, which is the crucial parameter for peptide-based vaccine development and which in most cases strongly correlates with the stability of the peptide-MHC interaction (van der Burg et al., 1996), the methods described by Sette et al., 1994, in combination with quantitative HLA-binding assays, may be used. Alternatively, immunogenicity of the selected peptide may be checked by performing *in vitro* CTL induction by known methods e.g. as described below for *ex vivo* CTL induction.

Alternatively to peptides derived from the naturally expressed tumor antigens, functional equivalents thereof, i.e. peptides with partially altered sequences or substances mimicking peptides, e.g. "peptidomimetics" or retro-inverso peptides, may be obtained by the following methods:

To enhance the immunogenicity of the peptides, amino acid substitutions may be introduced at anchor positions to increase peptide MHC class I-binding affinity. The modified peptides are subsequently evaluated for enhanced binding and immunogenicity by screening for recognition by TIL (tumor-infiltrating lymphocytes) and CTL induction as described by Parkhurst et al., 1996, and Bakker et al., 1997.

Another method useful in the present invention to find more immunogenic peptides by screening peptide libraries with a known CTL was described by Blake et al. 1996; it suggests the use of combinatorial peptide libraries for constructing functional mimics of tumor epitopes recognized by MHC class I-restricted CTLs.

In principle, the selection of peptides capable of eliciting a cellular immune response is carried out in several steps, as described in WO 97/30721, which disclosure is incorporated herein by reference. In short, the candidates are first tested for their binding ability to an MHC molecule; subsequently good binders are tested for immunogenicity. A general



strategy for obtaining efficient immunogenic peptides has been described by Schweighoffer, 1997.

The polypeptide of the present invention or immunogenic peptides derived from its sequence, respectively, can be produced recombinantly or by peptide synthesis, as described in WO 96/10413, the disclosure of which is incorporated herein by reference. For recombinant production, a DNA molecule encoding the antigen or the CTL peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell, cultivated under conditions suitable for expression, recovered and purified. For chemical synthesis, various conventional techniques may be used, e.g. commercially available automatic synthesizers.

The tumor antigen of the present invention and the immunogenic peptides derived therefrom or the respective peptide equivalents are useful in cancer therapy, e.g. to induce, in the context of the appropriate MHC presenting molecule, an immunological response to tumors which express the corresponding antigen determinants. The induction of CTLs can be accomplished *in vivo* or *ex vivo*.

For *in vivo* induction of CTLs, a pharmaceutical composition comprising the peptide/antigen is administered to an individual suffering from a tumor associated with the respective tumor antigen in an amount sufficient to elicit an effective CTL response to the antigen-bearing tumor. Thus, the present invention provides pharmaceutical compositions for therapeutic treatment which are intended for parenteral, topical, oral or local administration. Preferably, the compositions are for parenteral administration, e.g. for intravenous, subcutaneous, intradermal or intramuscular application. The peptides/antigens are dissolved or suspended in a pharmaceutically acceptable carrier, preferably an aqueous carrier. The composition may contain additional auxiliary substances, e.g. buffering agents, etc. The peptides may be used alone or in combination with adjuvants, e.g. saponins, alum, or, in a particularly preferred embodiment, polycations,

like polyarginine or polylysine. The peptides may also be linked to components assisting CTL priming, e.g. T helper peptides, lipids or liposomes or coadministered with such components or with immunostimulating substances, e.g. cytokines (IL-2, IFN- $\gamma$ ). Methods and compositions for preparing and administering pharmaceutical compositions for therapeutic treatments are described in WO 95/04542 and WO 97/30721 the disclosures of which are herein incorporated by reference.

The immunogenic peptides may also be used to elicit a CTL response *ex vivo*. An *ex vivo* CTL response to a tumor expressing the antigen is induced by incubating a patient's CTL precursor cells together with antigen presenting cells and the immunogenic peptide. The thus activated CTLs are allowed to mature and expand to effector CTLs which are then readministered to the patient. Alternatively, the tumor antigen may be pulsed onto APCs which present MHC class II-reactive peptides (Mayordomo et al., 1995; Zitvogel et al., 1996). A suitable method for loading peptides onto cells, e.g. dendritic cells, is disclosed in WO 97/19169.

The peptides of the invention are preferably applied as a combination of peptides, e.g. different CAMEL-peptides. In an even more preferred embodiment, the peptides of the invention are combined with peptides derived from other tumor antigens, e.g. LAGE-1 and ESO-NY-1. The selection of the peptides is optimized towards covering multiple HLA types in order to be useful for a broad population of patients and/or towards a broad variety of malignancies, which is taken into account by combining peptides from a large variety of tumor antigens. The number of peptides suitable to be combined to yield an efficient therapy may vary within a broad range, e.g. from about 2 to approximately 100.

In a further aspect, the present invention is directed to an isolated DNA molecule with the sequence set forth in SEQ ID NO: 1 encoding CAMEL.

This DNA molecule, which is designated "CAMEL-DNA", contains the ORF-1 of LAGE-1 cDNA which is defined by nucleotides 54 - 336 of the sequence set forth in SEQ ID NO: 3.

The CAMEL-DNA of the present invention, or the corresponding RNA, may  
5 be used, as an alternative to the use of the protein or the peptide, for cancer immunotherapy. Alternatively to using the natural sequence or fragments thereof, engineered derivatives may be utilized. These include sequences modified to encode (poly)peptides with improved immunogenicity, e.g. taking into account the modifications described above  
10 for the peptides. Another form of modification is the assembly of multiple sequences encoding immunologically relevant peptides in a so-called string-of-beads fashion, as described by Toes et al., 1997. The sequences may also be modified by adding auxiliary coding elements, e.g. targeting functions that ensure more efficient delivery and processing of the  
15 immunogen (e.g. Wu et al., 1995).

The nucleic acid molecules may be delivered either directly or as part of a recombinant virus or bacterium. In principle, any method that is known for gene therapy may be applied for nucleic acid-based cancer immunotherapy, both *in vivo* and *ex vivo*.

20 Examples for *in vivo* delivery are direct injection (injection of "naked" DNA) either intramuscularly or by "gene gun", which has been shown to result in the generation of CTLs against tumor antigens. Examples for recombinant organisms are vaccinia virus, fowlpox virus and adenovirus or Listeria monocytogenes (see Coulie, 1997 for a comprehensive review).  
25 Furthermore, synthetic nucleic acid carriers like cationic lipids, microspheres, microbeads, liposomes may be useful for *in vivo* delivery of the sequence encoding respective antigen/peptide. Similarly as for peptides, various auxiliary agents that enhance the immune response may be co-applied, e.g. cytokines, either as proteins or as plasmids encoding  
30 these.

Examples for *ex vivo* delivery are transfection of dendritic cells (Tuting, T., 1997) or other antigen presenting cells which are applied as a cellular cancer vaccine.

5 The present invention is also directed to the use of cells that express the tumor-associated antigen of the invention, either naturally or upon transfection with the respective coding sequence, for the preparation of a tumor vaccine.

10 In the present invention, it has been shown that CTL clones raised against IL-2 producing melanoma cell line 518/IL-2.14 are reactive against two alternatively spliced variants of LAGE-1, LAGE-1<sup>S</sup> (SEQ ID NO: 3) and LAGE-1<sup>L</sup> (SEQ ID NO: 5) and NY-ESO-1 (SEQ ID NO: 9). NY-ESO-1 is a recently described tumor antigen, identified by screening a cDNA library of an esophagus carcinoma with autologous patient serum (SEREX-method (Chen et al., 1997)). NY-ESO-1 is expressed in different tumor types but  
15 not in healthy tissues except the testis.

In the present invention, the epitope of specific CTL 1/29 was determined by cDNA expression cloning and a truncated LAGE-1 cDNA clone was found. This truncation led to the identification of the peptide epitope in an alternative reading frame, since the "normal" translation initiation site of  
20 LAGE-1 was absent. However, COS/HLA-A\*0201 cells transfected with full length LAGE-1 or NY-ESO-1 cDNA clones could stimulate the CTL clone to TNF- $\alpha$  production as well. This probably means that two different proteins can be translated from one single mRNA.

25 NY-ESO-1 also has been described as the target of melanoma-specific HLA-A\*0201 restricted CTL clones, which recognize an epitope translated in ORF3, located between aa 155 and 167 (Jäger et al., 1998). Therefore, it is very likely that also LAGE-1<sup>S</sup> will be recognized by these clones, but not LAGE-1<sup>L</sup>, since the protein sequence is different at this part of the molecule. The CAMEL-specific CTL clones recognize a peptide in an

alternative reading frame, which is encoded in both LAGE-1 and NY-ESO-1. This means that tumor cells expressing either LAGE-1 or NY-ESO-1 can be recognized by MLMAQEALAFI-specific CTL, which might enlarge the number of tumors that can be treated with immunotherapy based on this peptide.

Brief description of the Figures:

Figure 1: COS-7 transfection experiments with cDNA clone CAMEL and deletion constructs

- 10 a) COS-7 cells were transfected with cDNAs as indicated and tested with CTL 1/29 in a TNF- $\alpha$  release assay.
- b) Deletion constructs of CAMEL cDNA were cotransfected with HLA-A\*0201 cDNA in COS-7, followed by a TNF- $\alpha$  release assay with CTL 1/29. The PCR clones contain the numbers of nucleotides of the CAMEL cDNA as indicated.

Figure 2:

- a) Nucleotide alignment of cDNA clones CAMEL, LAGE-1<sup>S</sup>, LAGE-1<sup>L</sup> and NY-ESO-1.
- b) Protein translations of the cDNA clones LAGE-1<sup>S</sup>, LAGE-1<sup>L</sup> and NY-ESO-1. The translation of CAMEL is identical to the translation of LAGE-1<sup>S/L</sup> in ORF1. Although ORF3 seems the most putative one, the CTL epitope is encoded in ORF1 (underlined).

Figure 3: Characterisation of peptides recognized by CTL clone 1/29

- a) TNF- $\alpha$  release assay with predicted HLA-A\*0201 binding CAMEL peptides. Peptides as indicated were loaded on BLM, an

HLA-A\*0201<sup>+</sup> melanoma cell line, at a concentration of 10 µg/ml and tested with CTL 1/29 in a TNF-α release assay.

- b) The effects of increasing concentrations of peptides, derived from the major target epitope MLMAQEALAFI on recognition by CTL 1/29.
- 5 Various concentrations of peptides as indicated were loaded on HLA-A\*0201<sup>+</sup> cells and tested in a TNF-α release assay with CTL 1/29.

Figure 4: LAGE-1<sup>SL</sup> and NY-ESO-1 encode the CTL epitope

COS/HLA-A\*0201 cells were transfected with these cDNA clones and

10 reactivity with CTL 1/29 was measured in a TNF-α release assay.

Figure 5: His-tagged CAMEL protein, synthesized in E.coli

Figure 6: Expression of LAGE-1<sup>SL</sup> and NY-ESO-1 in healthy human tissues and melanoma cell lines

- a) Northern Blot analysis of the expression of LAGE-1/NY-ESO-1 in a
- 15 panel of healthy human tissues as indicated. The Blot was hybridised with <sup>32</sup>P-dCTP-labeled LAGE-1<sup>S</sup> cDNA.
- b) RT-PCR for LAGE-1 and NY-ESO-1. To discriminate between LAGE-1 and NY-ESO-1 mRNA, the same panel of melanoma cell
- 20 lines was analysed by RT-PCR with gene-specific primers. Melanoma cell lines as indicated were used as targets in a TNF-α release assay with CTL 1/29.

Figure 7: Immunohistochemical analysis of CAMEL expression in human tumors

Figure 8: Stabilization of HLA-A2 surface expression by synthetic

25 peptides on T2-cells

## Brief description of the sequences:

- SEQ ID NO: 1: CAMEL (4H8) cDNA sequence and translation
- SEQ ID NO: 2: CAMEL protein sequence
- 5 SEQ ID NO: 3: LAGE-1<sup>S</sup> cDNA sequence and translation
- SEQ ID NO: 4: LAGE-1<sup>S</sup> protein sequence
- SEQ ID NO: 5: LAGE-1<sup>L</sup> cDNA sequence and translation
- SEQ ID NO: 6: LAGE-1<sup>L</sup> protein sequence
- SEQ ID NO: 7: NY-ESO-1 cDNA sequence and translation
- 10 SEQ ID NO: 8: NY-ESO-1 protein sequence
- SEQ ID NO: 9: NY-ESO-1 cDNA and alternative translation
- SEQ ID NO: 10: protein sequence of alternatively translated NY-ESO-1
- SEQ ID NO: 11: peptide sequence of the CAMEL CTL epitope (11-mer)
- SEQ ID NO: 12: peptide sequence of the CAMEL CTL epitope (10-mer)
- 15 SEQ ID NO: 13: oligonucleotide SP6F-pSV
- SEQ ID NO: 14: oligonucleotide R1
- SEQ ID NO: 15: oligonucleotide R2
- SEQ ID NO: 16: oligonucleotide T7R-pSV
- SEQ ID NO: 17: oligonucleotide F3
- 20 SEQ ID NO: 18: oligonucleotide ESO-1B
- SEQ ID NO: 19: oligonucleotide ESO-1A
- SEQ ID NO: 20: oligonucleotide 4H8-A
- SEQ ID NO: 21: oligonucleotide 4H8-C
- SEQ ID NO: 22: oligonucleotide CAMEL-XHO
- 25 SEQ ID NO: 23: oligonucleotide CAMEL-KPN

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- SEQ ID NO: 24     peptide CAMEL10
- SEQ ID NO: 25     peptide CAMEL16
- SEQ ID NO: 26     peptide CAMEL17
- SEQ ID NO: 27     tyrosinase peptide
- 5   SEQ ID NO: 28     MAGE-3 peptide

In the Examples, if not stated otherwise, the following materials and methods were used

a) Cell cultures

- 10   Melanoma cell lines and COS-7 cells were maintained in DMEM containing 4.5 mM glucose supplemented with 8% FCS, 2 mM L-glutamine, 100 µg/ml of each penicillin and streptomycin. Melanoma cell line 518A2 was established from the dissected metastasis of a male patient in 1985, as described before (Versteeg et al., 1988). An IL-2 producing variant, 518/IL-
- 15   2.14, was obtained by transfection of 518A2 with the IL-2 cDNA (Osanto et al., 1993). Other melanoma cells that were used as targets in TNF-α release assay are FM3.29, FM6, FM28.4 and FM55P (gifts from J. Zeuthen, Denmark), MM127, MM415, MM485 (gifts from N. Hayward, Australia), SK-MEL-23, SK-MEL-29 (obtained from T. Wölfel, Mainz), Mi10221, Mi3046/2,
- 20   NA8, BLM (obtained from M. Visseren, Leiden). EBV-transformed B-LCL and the TNF-α-sensitive WEHI-164 clone 13 (a gift from Dr. P. Coulie, Brussels) were cultured in RPMI-1640, supplemented with L-glutamine and antibiotics as above, and 10% FCS.

- With the IL-2 producing cell line 518/IL-2.14 and autologous peripheral
- 25   blood mononuclear cells (PBMC) a CTL induction was performed, resulting in melanoma-specific HLA-A\*0201 restricted CTL clones (van Elsas et al., 1997). The identification of the epitope of one of these clones, CTL 1/29, is reported here.



**b) cDNA expression cloning**

A cDNA library of 518/IL-2.14 was constructed in the expression vector pSVsport1 (GIBCO, BRL) using the Superscript Plasmid System (GIBCO, BRL). As to that purpose, poly-A<sup>+</sup> mRNA was isolated using the Fast-Track system (Invitrogen), followed by reverse-transcription with an oligo-dT/NotI primer. Sall adapters were ligated to ds-cDNA and after NotI digestion and size fractionation, cDNA fragments were cloned into the pSVsport1 vector digested with Sall and NotI. After electroporation into ElectroMAX-DH10B (GIBCO, BRL) (following the manufacturers instructions) and selection for ampicillin resistance, 50-100 colonies were pooled for mini DNA isolation (QIAprep 8 plasmid kit, Qiagen). The in this way obtained cDNA pools were transfected in duplicate into COS-7 cells, together with the restriction element HLA-A\*0201 (pBJ1.neo/HLA-A\*0201, (Lin et al., 1990)), using the DEAE-dextran method. Briefly, COS-7 cells were seeded in 96-wells flatbottom plates at  $1.5 \times 10^4$  cells per well in 100  $\mu$ l DMEM, 8% FCS. After 2 hours, medium was replaced by 30  $\mu$ l transfection mixture, containing 100 ng cDNA pool, 100 ng HLA-A\*0201 cDNA, 400 ng/ml DEAE-dextran and 100  $\mu$ M chloroquine in serum free DMEM. Cells were incubated for 4 hours at 37°C and shocked for 2 minutes by the addition of 50  $\mu$ l 10% DMSO in PBS. The shock medium was replaced by 200  $\mu$ l DMEM, 8% FCS, and 48 hours later the cells were used as target cells for CTL in a TNF- $\alpha$  release assay.

**c) Deletion constructs**

Deletion constructs of cDNA clone 4H8 were obtained by PCR. PCR products were cloned in vector pCR3.uni (TA cloning system, Invitrogen). The constructs pCR-246 and pCR-464 were made with the vector-based forward primer, SP6F-pSV (SEQ ID NO: 13) and the reverse primers in cDNA 4H8, R1 (SEQ ID NO: 14) and R2 (SEQ ID NO: 15) respectively. As a control the complete 679 bp cDNA was cloned by PCR with two primers

on the pSVsport vector, SP6F-pSV (SEQ ID NO: 13) and T7R-pSV (SEQ ID NO: 16), resulting in pCR-679.

d) TNF- $\alpha$  release assay

CTL reactivity against tumor target cells, transfected COS-7 or peptide  
5 loaded cells was measured in a TNF- $\alpha$  release assay. Target cells were seeded in duplicate or triplicate at  $1.5\text{-}2 \times 10^4$  cells per well in a 96-wells flat bottom plate and 1500-2000 CTL were added to each well, in a total volume of 100  $\mu\text{l}$  / well (IMDM, supplemented with antibiotics and 5% FCS). After 24 hours of co-culturing of effector and target cells, 50  $\mu\text{l}$  out of each  
10 well was added to a fresh 96-wells flatbottom plate, containing 50  $\mu\text{l}$  ( $4.5 \times 10^4$ ) TNF- $\alpha$ -sensitive WEHI-164 cells per well in IMDM, supplemented with antibiotics, 5% FCS, 2  $\mu\text{g/ml}$  Actinomycin D and 40 mM LiCl. A viability staining was performed 24 hours later by the addition of 50  $\mu\text{l}$  of  
15 3-(4,5dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) solution (2.5 mg/ml in PBS). After incubation for 2-4 hours at 37°C the OD<sub>550-650</sub> was measured. TNF- $\alpha$  release in pg/ml was calculated from a standard with known TNF- $\alpha$  concentrations.

e) Northern Blot analysis

To determine expression in healthy tissues a Multiple Tissue Northern Blot  
20 was obtained commercially (Clontech). As a probe, LAGE-1 cDNA was used, labeled with  $\gamma\text{-}^{32}\text{P}\text{-dCTP}$  by use of the Mega-Prime Labeling kit (Amersham).

f) RT-PCR

cDNA synthesis was performed using oligo-dT and M-MLV reverse  
25 transcriptase (Promega). Primers used for LAGE-1 specific PCR were the F3 (SEQ ID NO: 17) and ESO-1B primer (SEQ ID NO: 18). ESO-1B was also used as a reverse primer in the NY-ESO-1-specific PCR, while ESO-1A (SEQ ID NO: 19) was the forward primer in this reaction (Chen et

al., 1997). Reactions were performed in a Biometra-Uno or -Trio programmed as follows: 5 minutes 95°C, 30 cycles of 1 min. 95°C, 1 min. 58°C, 1 min. 72°C, followed by 10 minutes 72°C.

g) Expression of CAMEL in E. Coli

- 5 A fragment containing the coding sequence of CAMEL was made by PCR with the following primers:

4H8-A: GAAGAACATATGCTGATGGCCCAGGAGGC (SEQ ID NO: 20)

4H8-C: TTAAAGATCTCAGAACCGCCCCTGGTCG (SEQ ID NO: 21)

- This fragment was digested with NdeI and BglII and cloned in the NdeI and  
10 BamHI sites of vector pET19b (Novagen, Madison, WI). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was transformed into BL21(DE3)pLysS E. coli bacteria (Novagen, Madison, WI). After culturing the bacteria at 30°C until an OD<sub>600</sub> = 0.5, IPTG (1 mM) was  
15 added to induce overexpression of the His-tagged CAMEL protein. Samples were taken at 0h and 4h after IPTG induction and lysates of these samples were tested on a Western Blot with the Penta-His Antibody (Qiagen) according to the Western and Colony Blot protocol of the supplier. The His-tagged protein was visualized using the SuperSignal Substrate  
20 system for Western blotting (Pierce, Rockford, US).

h) Preparation of anti-CAMEL antisera

Antibodies against the CAMEL protein were raised by immunizing two rabbits with three synthetic peptides derived from hydrophobic regions of this molecule :

- 25 F4: (K)GAMLAQERRVPRAAEV(K) (pos. 15-31 of SEQ ID NO: 2)  
A5: (K)GQQGPRGREEAPRGVRM(K) (pos. 36-52 of SEQ ID NO: 2)  
B5: (K)KRRMEGAPAGPGGRTAA(K) (pos. 58-73 of SEQ ID NO: 2)

(The lysine residues at both termini enable the peptides to be linked to KLH.)

For rabbit no. 1, 1 mg of each peptide was linked chemically to 2.5 mg of the carrier molecule KLH (Keyhole Limpet Hemocyanin) and after dialyzing, 0.8 mg of this protein in CFA (Complete Freund's Adjuvants) was weekly injected subcutaneously. Another rabbit (no. 2) was injected six times with the three peptides, not linked to KLH, following the scheme of 300 µg s.c. in CFA, 300 µg s.c. in IFA, 4x boost of 150 µg. The reactivity and specificity of the antisera were confirmed in ELISA and Western blot experiments. After four immunizations, antisera of both rabbits were reactive with the recombinant CAMEL protein synthesized in *E.coli*, but differed in their precise epitope: rabbit no. 1 produced antibodies against the CAMEL-B5 peptide, whereas the serum of rabbit no. 2 reacted with peptide F4. The antisera will further be referred to as "antiserum B5" and "antiserum F4".

j) Preparation of CAMEL-EGFP fusion proteins

The CAMEL coding sequence was fused to the *Aequorea victoria* -derived Green Fluorescent Protein (GFP). The CAMEL cDNA molecule was cloned into the pEGFP-N1 vector (Clontech), which contains a cDNA encoding the Enhanced, red shifted variant of GFP. In order to clone the cDNA molecule in frame with the EGFP cDNA and unidirectional, two primers were designed. The forward primer designated CAMEL-XHO (TTACTCGAGATGCTGATGGCCCAGG; SEQ ID NO: 22 ) covers the initiation codon ATG and contains an Xho1 site and the reverse primer CAMEL-KPN (AAGGTACCTTGAACCGCCCCTGGTCG; SEQ ID NO: 23) contains a mutation of the stop-codon and a Kpn1 site. The vector carrying the fusion construct was transfected into COS cells by calcium phosphate precipitation, protein lysates of the cells were used for Western blotting using CAMEL antisera against the CAMEL peptides B5 and F4, and anti-EGFP antibodies to detect the fusion protein according to standard protocols.

### Example 1

cDNA clone 4H8 (CAMEL) encodes the target for melanoma-specific CTL1/29

The antigenic epitope of melanoma-specific CTL 1/29 was identified by the  
5 expression of cDNA library 518/IL2.14 and the restriction element HLA-A\*0201 in COS-7 cells, followed by CTL screening in a TNF- $\alpha$  release assay. A positive pool of cDNAs was subcloned and clone 4H8, called CAMEL (SEQ ID NO: 1), was found to stimulate TNF- $\alpha$  release by the CTL to a similar extent as the original 518/IL2.14 cell line (Fig. 1). COS-7 cells or  
10 COS-7 cells transfected with HLA-A\*0201 or the 4H8 cDNA only were not recognized. The isolated 4H8 cDNA clone has a 679 bp insert, which shows strong homology with NY-ESO-1 (SEQ ID NO: 7), a tumor antigen originally identified as a target for humoral immune responses by serum screening methods (SEREX) (Chen et al., 1997). Colony hybridization of  
15 the cDNA library, using clone 4H8 as a probe resulted in the detection of 2 types of full length clones which were called LAGE-1<sup>S</sup> (SEQ ID NO: 3) and LAGE-1<sup>L</sup> (SEQ ID NO: 5) (Fig. 2a). LAGE-1<sup>L</sup> contains a 229 bp insertion at position 457, which has the consensus sequences for an intron, starting with a 5' GT and ending 3' AG. This indicates alternative splicing of  
20 LAGE-1 mRNA. However, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1 cDNA sequence.

### Example 2

The peptide epitope of CTL 1/29 is coded in an alternative reading frame of LAGE-1 or NY-ESO-1

25 To identify which peptide was recognized by CTL 1/29, deletion constructs of cDNA 4H8 were transfected in HLA-A\*0201<sup>+</sup> COS-7 cells and tested in a TNF- $\alpha$  release assay. CTL reactivity was measured with all constructs (Fig. 1b), indicating that the epitope was coded within the first 330 bp of

clone 4H8. An HLA-A\*0201 binding motif search was performed on the predicted protein sequence of that region (Drijfhout et al., 1995; D'Amato et al., 1995), presuming that the ATG at position 10 in 4H8 functions as the translation initiation site. Predicted strong binding peptides at regions 1-11, 2-11, 1-9, 10-18, 11-19, 16-25, 17-25, 49-57, 55-63 and 70-78 of the CAMEL protein sequence (Fig. 2b) were added to HLA-A\*0201<sup>+</sup> BLM melanoma cells, and tested for CTL reactivity in a TNF- $\alpha$  release assay (Fig. 3b).

At a peptide concentration of 10  $\mu$ g/ml only the N-terminal 11- and 10-mer peptides (M)LMAQEALAF<sub>L</sub> (SEQ ID NO: 11 and NO: 12) induced preponderant recognition by CTL 1/29 (Fig. 3a), indicating that the epitope recognized by the CTL is located in the first 11 amino acids of the CAMEL-encoded protein. Closer inspection of peptides derived of this N-terminal 11-mer in a peptide concentration dependent TNF- $\alpha$  release assay (Fig. 3b) revealed that the methionine at position 1 as well as the leucine at position 11 are of crucial importance for reconstituting CTL reactivity. Deletion of either of these amino acids leads to an at least 5 decades higher peptide concentration required for comparable TNF- $\alpha$  release. The only other peptide showing weak activity is the 3-11 MAQEALAF<sub>L</sub>. In contrast, the MLMAQEALA has no activity at all (Fig. 3b), suggesting that the C-terminal amino acids FL do significantly contribute to the recognition by the CTL.

### Example 3

#### Comparison of CAMEL, LAGE-1<sup>SL</sup>, NY-ESO-1

As already mentioned, cDNA clone 4H8 lacks the first 84 bp of the LAGE-1<sup>S</sup> sequence, which means that it is devoid of the initiation codon at position 54 of that sequence (Fig. 2a). The first possible translation initiation site in 4H8 corresponds with the ATG at position 94 of LAGE-1<sup>S</sup>, which is however, not in frame with the first ATG at position 54. Therefore, the protein translated from the 4H8 cDNA clone is different from the putative

LAGE-1 protein, since translation takes place in another reading frame (Fig. 2a and b). 4H8 encodes a protein of 109 amino acids (SEQ ID NO: 2) with a predicted molecular weight of 11.7 kD. The LAGE-1<sup>S</sup> protein translated from the first ATG will be a 180 aa protein of 18.2 kD (SEQ ID NO: 4), while the unspliced variant, LAGE-1<sup>L</sup>, encodes a 210 aa protein of 21.1 kD (SEQ ID NO: 6). NY-ESO-1 protein (SEQ ID NO: 8) is probably of the same size as LAGE-1<sup>S</sup>, but differs at 26 amino acids. However, if translation of LAGE-1<sup>SL</sup> starts at the second ATG, proteins will be translated in another reading frame and are in that case identical to the protein translated from cDNA 4H8. Alternative translation of NY-ESO-1 (SEQ ID NO: 9 and NO: 10) results in a shorter variant of this protein (58 amino acids), because of an earlier stop codon (Fig. 2b), which differs from the CAMEL protein sequence only in its last 5 amino acids (Fig. 2b).

It was examined whether cells transfected with the complete LAGE-1 (or NY-ESO-1) cDNA clones are able to stimulate CTL 1/29. Remarkably, COS/HLA-A\*0201 cells transfected with LAGE-1<sup>S</sup>, the alternatively spliced LAGE-1<sup>L</sup> (as well as with the NY-ESO-1) cDNA are able to stimulate CTL 1/29 (Fig. 4). This indicates that, at least in COS-7 cells, protein translation also starts from the second start codon at nucleotide 94 in LAGE-1<sup>S</sup>, notwithstanding the presence of the first ATG at position 54. Also in this case, this results in the "alternative reading frame" peptide, MLMAQEALAF<sub>L</sub>, recognized by CTL 1/29.

#### Example 4

##### Expression of CAMEL in E. Coli

To investigate whether CAMEL is indeed translated from the ORF-1 of the CAMEL (4H8) cDNA, the CAMEL cDNA (SEQ ID No: 1) was cloned in a bacterial expression vector (pET19b) (Studier et al., 1990). This vector contains a 6xHis-tag coding sequence, allowing detection of the His-tagged protein with an anti-His antibody. The pET19b-CAMEL construct was

transformed into E.coli and the bacteria were treated with IPTG to induce expression of the His-tagged CAMEL protein. Extracts were analyzed by Western blotting using the Penta-His antibody. Western blotting of a lysate shows a 15.5 kD protein, only slightly higher than the expected 14.5 kD of the His-tagged CAMEL protein after staining with a anti-His antibody (Fig. 5).

The CAMEL cDNA (SEQ ID No: 1) was cloned in pET19b and expressed in E.Coli. Lanes 1 and 2 represent the samples taken at 0h, lanes 3 and 4 at 4h after induction with IPTG. Because CAMEL might be an unstable protein, induction of protein expression was performed in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of PMSF (a protease inhibitor). At the left the positions of the molecular weight marker proteins are indicated.

#### Example 5

Expression of LAGE-1 and NY-ESO-1 in healthy human tissues and melanoma cell lines

Hybridisation of Multiple Tissue Northern blots containing RNA of healthy human tissues with the LAGE-1<sup>S</sup> cDNA showed high expression in testis and placenta and low, (but clear) expression in heart, skeletal muscle and pancreas (Fig. 6a). The positive signals exist of two bands, probably reflecting LAGE-1<sup>S</sup>/NY-ESO-1 (750 bp) and LAGE-1<sup>L</sup> (1000 bp).

Several melanoma cell lines were tested for expression of LAGE-1 and NY-ESO-1 by(Northern Blot analysis and) RT-PCR (Fig. 6b). Because of the strong homology between both genes, it is not possible to discriminate between LAGE-1 and NY-ESO-1 on Northern Blot. Therefore RT-PCR was performed with specific primers. In most cell lines a correlated expression of LAGE-1 and NY-ESO-1 was found; only cell line FM3.29 had expression of LAGE-1, but was negative for NY-ESO-1. Other cell lines expressed



either both or none of the two genes (Fig. 6b). There was a good correlation between the level of expression and the recognition by CTL 1/29 (Fig. 6b).

#### Example 6

##### 5 Determination of LAGE1/CAMEL expression of in human tumors by RT-PCR

In order to evaluate the percentage of LAGE1/CAMEL positive human tumors, individual tumor tissues from breast or lung cancer patients were subjected to RT-PCR, as described in the Method section f), using the LAGE1-specific primers F3 (SEQ ID NO: 17) and ESO-1B (SEQ ID  
10 NO: 18); reactions were performed in a Perkin Elmer 9600 thermocycler with 35 (instead of 30) cycles.

As shown in Table 1, approximately 50% (3/6) of the tested breast cancer specimens and 80% (10/12) of the lung cancer specimens were shown to be positive for LAGE1/CAMEL mRNA .

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Table 1:

| <i>Tumor type</i>         | <i>#</i>    | <i>LAGE1/CAMEL mRNA</i> |
|---------------------------|-------------|-------------------------|
|                           |             |                         |
| breast (ILC)              | 71526 / 85  | -                       |
| breast (ILC)              | 44231 / 95  | -                       |
| breast (ILC)              | 73507 / 95  | +                       |
| breast (IDC)              | 19837 / 95  | ++                      |
| breast (IDC)              | 4385 / 95   | +                       |
| breast (IDC)              | 48897 / 95  | -                       |
|                           |             |                         |
| lung (AC)                 | 15827 / 97  | +                       |
| lung (AC)                 | 53934 / 97  | ++                      |
| lung (AC)                 | 67086 / 93  | ++                      |
| lung (AC)                 | 62357 / 96  | +                       |
| lung (AC)                 | T63244 / 93 | -                       |
| lung (AC)                 | T64360 / 93 | +                       |
|                           |             |                         |
| lung (SCC)                | 92710 / 96  | ++                      |
| lung (SCC)                | 53005       | ++                      |
| lung (SCC)                | 28649 / 97  | -                       |
| lung (SCC)                | 16251 / 97  | +                       |
| lung (SCC)                | 5063 / 93   | ++                      |
| lung (SCC)                | 7580 / 97   | +                       |
|                           |             |                         |
| testis (positive control) | (GibcoBRL)  | +                       |
|                           |             |                         |

### Example 7

#### Immunohistochemical analysis of CAMEL expression in human tumors

As described in Examples 5 and 6, LAGE-1 mRNA was detected in a panel  
5 of tumor cell lines and tumor tissues and in a restricted number of healthy  
tissues by means of RT-PCR and Northern blot experiments. However, it  
remained to be determined whether in cells expressing the LAGE-1 mRNA  
also the alternatively translated CAMEL is produced.

10 Frozen sections from a panel of different human tumors were analyzed by  
immunohistochemistry using a CAMEL-specific rabbit antiserum B5 which  
was affinity-purified against the B5 peptide. (For the preparation of the  
antisera, see h) in the Methods section. The B5 antiserum was used  
because B5 is CAMEL-specific, while the F4 antiserum may also recognize  
an epitope present on a protein expressed from the ORF-1 of NY-ESO-1).  
15 Specificity of the purified serum was demonstrated by peptide ELISA and  
Western blotting against COS cells transfected with a CAMEL-GFP fusion  
protein. Immunohistochemistry was performed using a 3-step avidin-biotin-  
peroxidase staining procedure. The results are summarized in Table 2,  
examples are shown in Figure 7.

Table 2:

| Tumor type<br>(n=number of cases) | Percentage of positive tumor cells |       |        |        |      |
|-----------------------------------|------------------------------------|-------|--------|--------|------|
|                                   | negative                           | < 10% | 10-40% | 41-70% | >70% |
| Breast AC (n=11)                  | 2                                  | 4     | 1      | 2      | 2    |
| Colon AC (n=8)                    | 1                                  | 0     | 1      | 1      | 5    |
| Lung AC (n=10)                    | 2                                  | 2     | 2      | 3      | 1    |
| Lung SCC (n=9)                    | 4                                  | 3     | 1      | 1      | 0    |
| Pancreas AC (n=10)                | 1                                  | 2     | 0      | 2      | 5    |

AC: adenocarcinoma

SCC: squamous cell carcinoma

5

A total of 48 different specimen was investigated. In the majority of cases (38/48) CAMEL expression was detected. About half of the positive cases showed expression of CAMEL in 40% or more of the tumor cells, in some of the cases close to 100% of the tumor cells showed CAMEL-specific staining (an example is shown Figure 7, Colon AC). In the majority of tumor specimens expression was heterogeneous ranging from less than 10% of positive tumor cells to more than 70% of positive tumor cells (Table 2; examples are shown in Figure 7, arrows indicate positive tumor cell staining).

10

15 Example 8

#### Identification of HLA-A2 binding peptides within the CAMEL ORF

In order to identify further HLA-A2 epitopes besides the CTL-epitope (M)LMAQEALAFI (SEQ ID NO:11 and 12), CAMEL (SEQ ID NO:2) was examined according to the algorithms and motifs published by Parker et al.;

1994, and Rammensee et al., 1995. The result of this analysis indicated that several further peptides within the CAMEL protein have the potential to bind to HLA-A2, and three of these candidates -CAMEL10: FLMAQGAML (SEQ ID NO: 24), CAMEL16: AMLAAQERRV (SEQ ID NO: 25) and  
5 CAMEL17: MLAAQERRV (SEQ ID NO:26) – were synthesized.

These synthetic peptides were evaluated for their ability to increase surface HLA-A2 expression on the transport defective cell line 174CEM.T2 (Nijman et al., 1993). Briefly,  $5 \times 10^5$  cells/0.2 ml/well were seeded in 96-well V-bottom plates and incubated for 16 hours with increasing amounts  
10 (0-320 µg/ml) of peptide at 37°C in a humidified atmosphere. HLA-A2 surface expression was measured by FACS analysis (Becton Dickinson) using purified BB7.2 as primary antibody and a goat-anti-mouse IgG RPE conjugate(DAKO) as detection antibody.

As positive controls the known HLA-A2 restricted CTL-epitopes from  
15 CAMEL (MLMAQEALAFI, SEQ ID NO:11) or tyrosinase (Wölfel et al., 1994; YMNGTMSQV, SEQ ID NO:27) were applied. Negative controls included an HLA-A1 binding (and therefore irrelevant) peptide from MAGE-3 (Gaugler et al. , 1994; EVDPIGHLY, SEQ ID NO:28) or no peptide at all.

20 The results from these experiments suggest that the nonamer CAMEL10 binds with similar affinity to HLA-A2 as compared to the positive controls used in the assay. The two other peptides (CAMEL16 and CAMEL17) showed only low affinity in this assay. Therefore in particular CAMEL10 represents a potential new HLA-A2 restricted CTL-epitope derived from  
25 CAMEL protein (FIG. 8).

The testing of the immunogenicity of CAMEL10 and if it represents a naturally processed and presented ligand can be done as described in WO 97/30721 and Schweighoffer, 1997.

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tumor-associated cytotoxic T lymphocyte epitopes in a string-of-beads  
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"self" antigens induce antitumor immunity *in vivo*. *Eur. J. Immunol.* 27,  
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Lotze, M.T., and Storkus, W.J. (1996). Therapy of murine tumors with tumor  
peptide-pulsed dendritic cells: dependence on T cells, B7 costimulation,  
and T helper cell 1-associated cytokines. *J. Exp. Med.* 183, 87-97.

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## SEQUENCE LISTING

5

## (1) GENERAL INFORMATION:

10

## (i) APPLICANT:

- (A) NAME: Boehringer Ingelheim International GmbH
- (B) STREET: Binger Strasse 173
- (C) CITY: Ingelheim am Rhein
- (E) COUNTRY: Germany
- (F) POSTAL CODE (ZIP): 55216
- (G) TELEPHONE: 06132/772282
- (H) TELEFAX: 06132/774377

15

20

## (ii) TITLE OF INVENTION: Tumor-associated Antigen

## (iii) NUMBER OF SEQUENCES: 28

25

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

30

35

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 679 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40

45

## (ii) MOLECULE TYPE: cDNA to mRNA

## (iii) HYPOTHETICAL: NO

## (iv) ANTI-SENSE: NO

50

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: homo sapiens
- (F) TISSUE TYPE: Melanoma

55

## (ix) FEATURE:

- (A) NAME/KEY: 3'UTR

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(B) LOCATION:340..679

(ix) FEATURE:

5 (A) NAME/KEY: 5'UTR  
(B) LOCATION:1..9

(ix) FEATURE:

10 (A) NAME/KEY: CDS  
(B) LOCATION:10..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

|    |   |     |
|----|---|-----|
| 15 | CGACGGGCG ATG CTG ATG GGC CAG GAG GGC CTG GCA TTC CTG ATG GGC   | 48  |
|    | Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala             |     |
|    | 1 5 10  |     |
| 20 | CAG GGG GCA ATG CTG GCG GGC CAG GAG AGG CCG GTG CCA CCG GCG GCA | 96  |
|    | Gln Gly Ala Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala |     |
|    | 15 20 25  |     |
| 25 | GAG GTC CCG GGG GCG CAG GGG CAG CAA GGG CCT CCG GGC CGA GAG GAG | 144 |
|    | Glu Val Pro Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu |     |
|    | 30 35 40 45   |     |
| 30 | GCG CCG CCG GGG GTC CCG ATG GCG GTG CCG CTT CTG CCG AGG ATG GAA | 192 |
|    | Ala Pro Arg Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu |     |
|    | 50 55 60  |     |
| 35 | GGT GGC CCT GCG GGG CCA GGA GGC CCG ACA GGC GGC TGC TTC AGT TGC | 240 |
|    | Gly Ala Pro Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys |     |
|    | 65 70 75  |     |
| 40 | ACA TCA CGA TGC CTT TCT CGT CCG CCA TGG AAG CCG AGC TGG TCC GCA | 288 |
|    | Thr Ser Arg Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala |     |
|    | 80 85 90  |     |
| 45 | GGA TCC TGT CCG GGG ATG CCG CAC CTC TCC CCG GAC CAG GGG CCG TTC | 336 |
|    | Gly Ser Cys Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe |     |
|    | 95 100 105  |     |
| 50 | TGA AGGACTTCAC CGTGTCGGC AACCTACTGT TTATCCGACT GACTGCTGCA       | 389 |
|    | *   |     |
| 55 | 110   |     |
|    | GACCAACGCG AACTGCAGCT CTCATCAGC TCTGTCTCC AGCAGCTTC CCGTTCATG   | 449 |
|    | TGGATCAGC AGTGCCTTCT GCGGTGTTT TTGGCTCAGG CTCCCTCAGG GCAGAGGCGC | 509 |
|    | TAAGCCACG CTGGGGGCC TTCTAGGTC ATGCTCTCTC CCTAGGGAA TGGTCCACG    | 569 |
|    | ACGAGGGGC AGTTCATGT GGGGGCTGA TTGTTGTGCG CTGAGGAGG ACGGCTTACA   | 629 |
|    | TGTTGTGTTT TGTAGAAAT AAGCTGAGC TAGGAAAAA AAAAAAAAAA             | 679 |

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## (2) INFORMATION FOR SEQ ID NO: 2:

## 5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 109 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

15 Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala  
 1 5 10 15  
 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
 20 25 30  
 20 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
 35 40 45  
 Gly Val Arg Met Ala Val Pro Leu Leu Arg Arg Met Glu Gly Ala Pro  
 50 55 60  
 25 Ala Gly Pro Gly Gly Arg Thr Ala Ala Cys Phe Ser Cys Thr Ser Arg  
 65 70 75 80  
 30 Cys Leu Ser Arg Arg Pro Trp Lys Arg Ser Trp Ser Ala Gly Ser Cys  
 85 90 95  
 Pro Gly Met Pro His Leu Ser Pro Asp Gln Gly Arg Phe \*  
 100 105 110

35

## (2) INFORMATION FOR SEQ ID NO: 3:

## 40 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 767 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

50 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: homo sapiens

(F) TISSUE TYPE: Melanoma

55

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 54..596

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(ix) FEATURE:

(A) NAME/KEY: 3'UTR

(B) LOCATION: 597..767

5

(ix) FEATURE:

(A) NAME/KEY: 5'UTR

(B) LOCATION: 1..53

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

|    |   |     |
|----|---|-----|
| 15 | ATCTCTGGTGG GGGCTGACCT TCCTCTGAG AGGCGGCGAG AGGCTCCGGA GGC ATG  | 56  |
|    | Met   |     |
|    | 1   |     |
| 20 | CAG GGC GAA GGC CAG GGC ACA GGG GGT TOG AOG GGC GAT GCT GAT GGC | 104 |
|    | Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly |     |
|    | 5 10 15   |     |
| 25 | CCA GGA GGC OCT GGC ATT OCT GAT GGC CCA GGG GGC AAT GCT GGC GGC | 152 |
|    | Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly |     |
|    | 20 25 30  |     |
| 30 | CCA GGA GAG GGG GGT GGC ACG GGC GGC AGA GGT CCC CCG GGC GCA GGC | 200 |
|    | Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly |     |
|    | 35 40 45  |     |
| 35 | GCA GCA AGG GGC TOG GGG CCG AGA GGA GGC GGC CCG CCG GGT CCG CAT | 248 |
|    | Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His |     |
|    | 50 55 60 65   |     |
| 40 | GGC GGT GGC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GGC AGG | 296 |
|    | Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg |     |
|    | 70 75 80  |     |
| 45 | AGG CCG GAC AGC CCC CTG CTT CAG TTG CAC ATC ACG ATG OCT TTC TOG | 344 |
|    | Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser |     |
|    | 85 90 95  |     |
| 50 | TOG CCC ATG GAA GCG GAG CTG GTC GGC AGG ATC CTG TOC CCG GAT GGC | 392 |
|    | Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala |     |
|    | 100 105 110   |     |
| 55 | GCA OCT CTC CCC CGA CCA GGG GGG GTT CTG AAG GAC TTC ACC GTG TOC | 440 |
|    | Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser |     |
|    | 115 120 125   |     |
| 60 | GGC AAC CTA CTG TTT ATC CGA CTG ACT GCT GCA GAC CAC CCG CAA CTG | 488 |
|    | Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln Leu |     |
|    | 130 135 140 145   |     |
| 65 | CAG CTC TOC ATC AGC TOC TGT CTC CAG CAG CTT TOC CTG TTG ATG TGG | 536 |
|    | Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met Trp |     |
|    | 150 155 160   |     |

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ATC AGC CAG TGC TTT CTG CCC GTG TTT TTG GCT CAG GCT CCC TCA GGG 584  
 Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser Gly  
 165 170 175

5 CAG AGG CGC TAA GGGCAGCTG GGGGGGCTC CTAGGTCATG CCTCTCTCCC 636  
 Gln Arg Arg \*  
 180

10 TAGGGAATGG TOCCAGCAAG AGTGGCCAGT TCATTGTGGG GGCTGATTG TTGTGCGCTG 696  
 CAGGAGGACG GCTTACATGT TTGTTCTGT AGAAATATAA GCTGAGCTAC GAAAAAAAAA 756  
 AAAAAAAAAA A 767

15

## (2) INFORMATION FOR SEQ ID NO: 4:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 180 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

30 Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp  
 1 5 10 15

Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly  
 20 25 30

35 Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala  
 35 40 45

Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro  
 50 55 60

40 His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala  
 65 70 75 80

45 Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe  
 85 90 95

Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp  
 100 105 110

50 Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val  
 115 120 125

Ser Gly Asn Leu Leu Phe Ile Arg Leu Thr Ala Ala Asp His Arg Gln  
 130 135 140

55 Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met  
 145 150 155 160



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Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Ala Pro Ser  
 165 170 175

5 Gly Gln Arg Arg \*  
 180

## (2) INFORMATION FOR SEQ ID NO: 5:

10

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 993 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

20

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

25

- (A) ORGANISM: homo sapiens  
 (F) TISSUE TYPE: Melanoma

(ix) FEATURE:

- (A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..55

30

(ix) FEATURE:

- (A) NAME/KEY: CDS  
 (B) LOCATION:56..688

35

(ix) FEATURE:

- (A) NAME/KEY: 3'UTR  
 (B) LOCATION:689..993

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCATCTCTGT GGGGCTGAC CTCCTCTCTG AGAGCGGGC AGAGGCTTGG GAGGC ATG 58  
 Met  
 1

45

CAG GGC GAA GGC CAG GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC 106  
 Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp Gly  
 5 10 15

50

CCA GGA GGC OCT GGC ATT OCT GAT GGC CCA GGC GGC AAT GCT GGC GGC 154  
 Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly  
 20 25 30

55

CCA GGA GAG GCG GGT GGC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGC 202  
 Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly  
 35 40 45

GCA GCA AGG GGC TGG GGG CCG AGA GGA GGC GGC CCG CGG GGT CCG CAT 250

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|    |  |      |
|----|--|------|
|    | Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro His  |      |
|    | 50 55 60 65  |      |
| 5  | GGC GGT GGC GCT TCT GCG CAG GAT GGA AGG TGC CCC TGC GGG GGC AGG<br>Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala Arg | 298  |
|    | 70 75 80   |      |
| 10 | AGG CCG GAC AGC GCG CTG CTT CAG TTG CAC ATC ACG ATG OCT TTC TCG<br>Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe Ser | 346  |
|    | 85 90 95   |      |
| 15 | TCG CCC ATG GAA GCG GAG CTG GTC GCG AGG ATC CTG TCC CCG GAT GGC<br>Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp Ala | 394  |
|    | 100 105 110  |      |
| 20 | GCA OCT CTC CCC CGA CCA GGG GCG GTT CTG AAG GAC TTC ACC GTG TCC<br>Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val Ser | 442  |
|    | 115 120 125  |      |
| 25 | GGC AAC CTA CTG TTT ATG TCA GTT CCG GAC CAG GAC AGG GAA GGC GCT<br>Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly Ala | 490  |
|    | 130 135 140 145  |      |
| 30 | GGG CCG ATG AGG GTG GTG GGT TGG GGG CTG GGA TCC GGC TCC CCG GAG<br>Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro Glu | 538* |
|    | 150 155 160  |      |
| 35 | GGG CAG AAA GCT AGA GAT CTC AGA ACA CCC AAA CAC AAG GTC TCA GAA<br>Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser Glu | 586  |
|    | 165 170 175  |      |
| 40 | CAG AGA OCT GGT ACA CCA GGC CCG CCG CCA CCC GAG GGA GGC CAG GGA<br>Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Pro Glu Gly Ala Gln Gly | 634  |
|    | 180 185 190  |      |
| 45 | GAT GGG TGC AGA GGT GTC GGC TTT AAT GTG ATG TTC TCT GGC OCT CAC<br>Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro His | 682  |
|    | 195 200 205  |      |
| 50 | ATT TAG CCGACTGACT GCTGCAGAAC ACGGCCAACT GCAGCTCTCC ATCAGCTCCT<br>Ile *  | 738  |
|    | 210  |      |
| 55 | GTCTCAGCA GCTTTCCCTG TTGATGIGGA TCAGGCAGTG CTTTCIGGCC GTGTATTGG  | 798  |
|    | CTCAGGCTCC CTCAGGGCAG AGGGGCTAAG CCCAGCCTGG CGCCCCCTCC TAGGTATGC   | 858  |
|    | CTCTCCCTT AGGGAATGT CCAGCAAGA GTGGCCAGTT CATTGIGGGG GCTGATTTG  | 918  |
|    | TTGTGCTGG AGGAGGAGG CTACATGTT TGTTCIGTA GAAATTAAG CTAGCTAAG  | 978  |
|    | AAAAAAAA AAAAA   | 993  |

(2) INFORMATION FOR SEQ ID NO: 6:

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## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 210 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

10 Met Gln Ala Glu Gly Gln Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp  
       1                  5                  10                  15  
       Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly  
                   20                  25                  30  
 15 Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala  
                   35                  40                  45  
       Gly Ala Ala Arg Ala Ser Gly Pro Arg Gly Gly Ala Pro Arg Gly Pro  
 20                  50                  55                  60  
       His Gly Gly Ala Ala Ser Ala Gln Asp Gly Arg Cys Pro Cys Gly Ala  
                   65                  70                  75                  80  
 25 Arg Arg Pro Asp Ser Arg Leu Leu Gln Leu His Ile Thr Met Pro Phe  
                   85                  90                  95  
       Ser Ser Pro Met Glu Ala Glu Leu Val Arg Arg Ile Leu Ser Arg Asp  
                   100                  105                  110  
 30 Ala Ala Pro Leu Pro Arg Pro Gly Ala Val Leu Lys Asp Phe Thr Val  
                   115                  120                  125  
       Ser Gly Asn Leu Leu Phe Met Ser Val Arg Asp Gln Asp Arg Glu Gly  
 35                  130                  135                  140  
       Ala Gly Arg Met Arg Val Val Gly Trp Gly Leu Gly Ser Ala Ser Pro  
                   145                  150                  155                  160  
 40 Glu Gly Gln Lys Ala Arg Asp Leu Arg Thr Pro Lys His Lys Val Ser  
                   165                  170                  175  
       Glu Gln Arg Pro Gly Thr Pro Gly Pro Pro Pro Pro Glu Gly Ala Gln  
                   180                  185                  190  
 45 Gly Asp Gly Cys Arg Gly Val Ala Phe Asn Val Met Phe Ser Ala Pro  
                   195                  200                  205  
       His Ile \*  
 50                  210

55

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 752 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens

15 (ix) FEATURE:  
 (A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..53

20 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION:54..596

25 (ix) FEATURE:  
 (A) NAME/KEY: 3'UTR  
 (B) LOCATION:597..752

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

|    |   |     |
|----|---|-----|
| 30 | ATCTCTGTGG GGCCTGACCT TCCTCTGAG AGCGGGCAG AGGCTCCGA GGC ATG     | 56  |
|    | Met   |     |
|    | 1   |     |
| 35 | CAG GGC GAA GGC CGG GGC ACA GGG GGT TGG ACG GGC GAT GCT GAT GGC | 104 |
|    | Gln Ala Glu Gly Arg Gly Thr Gly Ser Thr Gly Asp Ala Asp Gly     |     |
|    | 5 10 15   |     |
| 40 | CCA GGA GGC CCT GGC ATT CCT GAT GGC CCA GGG GGC AAT GCT GGC GGC | 152 |
|    | Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly Gly |     |
|    | 20 25 30  |     |
| 45 | CCA GGA GAG GCG GGT GGC ACG GGC GGC AGA GGT CCC CGG GGC GCA GGC | 200 |
|    | Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala Gly |     |
|    | 35 40 45  |     |
| 50 | GCA GCA AGG GGC TGG GGG CCG GGA GGA GGC GGC CCG CGG GGT CCG CAT | 248 |
|    | Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro His |     |
|    | 50 55 60 65   |     |
| 55 | GGC GGC GCG GCT TCA GGG CTG AAT GGA TGC TGC AGA TGC GGC GGC AGG | 296 |
|    | Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala Arg |     |
|    | 70 75 80  |     |
| 55 | GGG CCG GAG AGC CGC CTG CTT GAG TTC TAC CTC GGC ATG OCT TTC GCG | 344 |
|    | Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe Ala |     |
|    | 85 90 95  |     |
|    | ACA CCC ATG GAA GCA GAG CTG GGC CGC AGG AGC CTG GGC CAG GAT GGC | 392 |

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Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp  
 100 105 110

5 Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val  
 115 120 125

Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln  
 130 135 140

10 Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met  
 145 150 155 160

Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser  
 165 170 175

15 Gly Gln Arg Arg \*

180

20

## (2) INFORMATION FOR SEQ ID NO: 9:

- 25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 752 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- 30 (ii) MOLECULE TYPE: cDNA to mRNA
- (iii) HYPOTHETICAL: NO
- 35 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: homo sapiens
- 40 (ix) FEATURE:  
 (A) NAME/KEY: 5'UTR  
 (B) LOCATION:1..93
- 45 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION:94..270
- (ix) FEATURE:  
 (A) NAME/KEY: 3'UTR  
 50 (B) LOCATION:271..752

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

55 ATCTCTGTGG GCGCTGACCT TCTCTCTGAG AGCGGGGAG AGGCTGCGGA GGCATGCAGG 60

CGGAGGCGG GGGCAGAGGG GGTGTGAGG GCG ATG CTG ATG GCG CAG GAG GCG 114

Met Leu Met Ala Gln Glu Ala

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|    |  |   |   |     |
|----|--|---|---|-----|
|    |  | 1 | 5 |     |
|    | CTG GCA TTC CTG ATG GGC CAG GGG GCA ATG CTG GCG GGC CAG GAG AGG  |   |   | 162 |
| 5  | Leu Ala Phe Leu Met Ala Gln Gly Ala Met Leu Ala Ala Gln Glu Arg  |   |   |     |
|    | 10 15 20   |   |   |     |
|    | CGG GTG CCA CCG GCG GCA GAG GTC CCC GGG GCG CAG GGG CAG CAA GGG  |   |   | 210 |
|    | Arg Val Pro Arg Ala Ala Glu Val Pro Gly Ala Gln Gly Gln Gln Gly  |   |   |     |
| 10 | 25 30 35   |   |   |     |
|    | OCT CCG GGC CCG GAG GAG GCG CCC CCG GGG GTC CCG ATG GCG GCG CCG  |   |   | 258 |
|    | Pro Arg Gly Arg Glu Glu Ala Pro Arg Gly Val Arg Met Ala Ala Arg  |   |   |     |
|    | 40 45 50 55  |   |   |     |
| 15 | CCT CAG GGC TGA ATGGATGCTG CAGATGCGGG GGCAGGGGGC CCGAGAGCGG      |   |   | 310 |
|    | Leu Gln Gly *  |   |   |     |
|    | OCTGCTTGAG TTCTAAGCTG CCATGCCCTT CGGACACCC ATGGAGCAG AGCTGGCCCG  |   |   | 370 |
| 20 | CAGGAGCCTG GGCAGGATG CCGCAGCGCT TCCCGTGCCA GGGGTGCTTC TGAAGGAGTT |   |   | 430 |
|    | CACGTGTGCC GGCACATAC TGACTATCG ACTGACTGCT GCAGACACC GCGAAGTCCA   |   |   | 490 |
| 25 | GCTCTCATC AGCTCTGTC TCCAGCAGCT TCCCTGTGTG ATGTGGATCA CCGAGTGCCT  |   |   | 550 |
|    | TCTGCGCGTG TTTTGGCTC AGCTCCCTC AGGGCAGAGG CCGTACGCC AGCTGGCGC    |   |   | 610 |
|    | CCCTTCTAG GTCATGCTC CTTCCCTAGG GAATGGTCC AGCAGAGTG CCGAGTTCAT    |   |   | 670 |
| 30 | TGCGGGGCG TGATTGTTG TCGTGGAGG AGGAGCGCTT ACATGTTGT TTCTGTAGAA    |   |   | 730 |
|    | AATAAACTG AGCTACAAA AA   |   |   | 752 |
| 35 |  |   |   |     |

## (2) INFORMATION FOR SEQ ID NO: 10:

- 40 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 58 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- 45 (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu Met Ala Gln Gly Ala  
 1 5 10 15  
 5 Met Leu Ala Ala Gln Glu Arg Arg Val Pro Arg Ala Ala Glu Val Pro  
 20 25 30  
 10 Gly Ala Gln Gly Gln Gln Gly Pro Arg Gly Arg Glu Glu Ala Pro Arg  
 35 40 45  
 Gly Val Arg Met Ala Ala Arg Leu Gln Gly \*
 50 55

15

2) INFORMATION FOR SEQ ID NO: 11:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 11 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

30 Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
 1 5 10....

35 2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 10 amino acids  
 (B) TYPE: amino acid  
 40 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

45 Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
 1 5 10.

50

(2) INFORMATION FOR SEQ ID NO: 13:

55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear



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(ii) MOLECULE TYPE: synthetic DNA

5 GGTCACACTA TAGAAGGTAC G 21

(2) INFORMATION FOR SEQ ID NO: 14:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

20 TGATGTGCAA CTGAAGCAGG.....20

25 (2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

35 GCACTGGGIG ATCCACATCA A 21

40 (2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

45

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic DNA

50 CGACTCACTA TAGGGAGAGA G 21

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## (2) INFORMATION FOR SEQ ID NO: 17:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: synthetic DNA

GCACATCAG ATGCGTTTCT CGTGG

25

15

## (2) INFORMATION FOR SEQ ID NO: 18:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 25 (ii) MOLECULE TYPE: synthetic DNA

CACACAAAGC TTGGCTTAGC GGCCTGCGC TG.....32

30

## (2) INFORMATION FOR SEQ ID NO: 19:

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 30 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 40 (ii) MOLECULE TYPE: synthetic DNA

CACACAGGAT CCATGGATGC TGCAGATGG.....30

45

## (2) INFORMATION FOR SEQ ID NO: 20:

## (i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 29 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 55 (ii) MOLECULE TYPE: synthetic DNA

GAGACATA TGCTGATGCC CCAGGAGGC

29

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## (2) INFORMATION FOR SEQ ID NO: 21:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 28 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 10 (ii) MOLECULE TYPE: synthetic DNA

TTAAAGATCT CAGAACCGCC OCTGGTGG

28

15

## (2) INFORMATION FOR SEQ ID NO: 22:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 25 (ii) MOLECULE TYPE: synthetic DNA

ttactogaga tgctgatggc ccagg.....25

30

## (2) INFORMATION FOR SEQ ID NO: 23:

## (i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

## 40 (ii) MOLECULE TYPE: synthetic DNA

aaggtaacctt gaacggccccc tggtag .....26

45

## 2) INFORMATION FOR SEQ ID NO: 24:

## (i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Phe Leu Met Ala Gln Gly Ala Met Leu  
 1 5 9

5

2) INFORMATION FOR SEQ ID NO: 25:

10

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 10 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

15

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Ala Met Leu Ala Ala Gln Glu Arg Arg Val  
 1 5 10

25

2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 9 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

30

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Met Leu Ala Ala Gln Glu Arg Arg Val  
 1 5 9

40

2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 10 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Tyr Tyr Met Asn Gly Thr Met Ser Gln Val  
 1 5 10

55

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## 2) INFORMATION FOR SEQ ID NO: 28:

## (i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 9 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Glu Val Asp Pro Ile Gly His Leu Tyr  
1 5 9

15

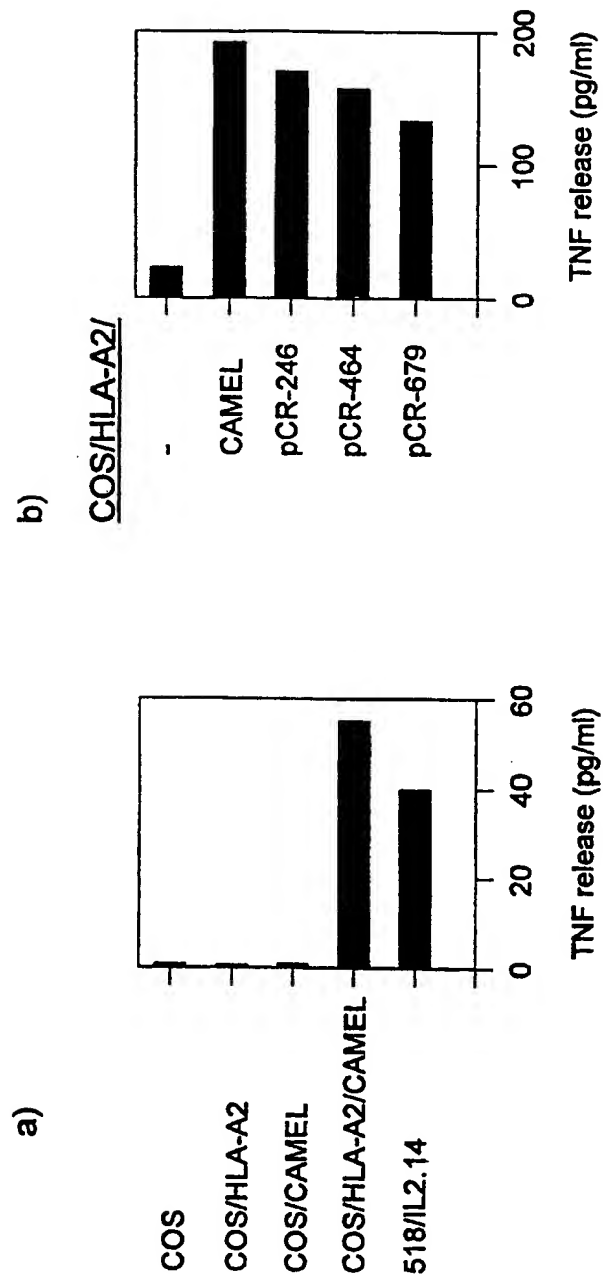
## Claims

1. Tumor-associated antigen CAMEL which is encoded by the ORF-1 of  
LAGE-1 cDNA and has the amino acid sequence set forth in  
5 SEQ ID NO: 2.
2. The tumor-associated antigen of claim 1 for use in cancer therapy.
3. Immunogenic (poly)peptide derived from the tumor-associated antigen  
of claim 1.
4. An immunogenic peptide of claim 3, characterized in that it has the  
10 amino acid sequence Met Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
(SEQ ID NO: 11).
5. An immunogenic peptide of claim 3, characterized in that it has the  
amino acid sequence Leu Met Ala Gln Glu Ala Leu Ala Phe Leu  
(SEQ ID NO: 12).
- 15 6. An immunogenic peptide of claim 3, characterized in that it has the  
amino acid sequence Phe Leu Met Ala Gln Gly Ala Met Leu  
(SEQ ID NO: 24).
7. An immunogenic peptide of claim 3, characterized in that it has the  
amino acid sequence Ala Met Leu Ala Ala Gln Glu Arg Arg Val  
20 (SEQ ID NO: 25).
8. An immunogenic peptide of claim 3, characterized in that it has the  
amino acid sequence Met Leu Ala Ala Gln Glu Arg Arg Val  
(SEQ ID NO: 26).
9. An immunogenic peptide of any one of claims 3 to 8 for use in cancer  
25 immunotherapy.
10. Pharmaceutical composition containing the tumor-associated antigen  
CAMEL of claim 1.

11. Pharmaceutical composition containing an immunogenic peptide of any one of claims 3 to 8.
12. Isolated DNA molecule comprising the sequence set forth in SEQ ID NO: 1.
- 5 13. Recombinant DNA molecule comprising the DNA molecule of claim 12.
14. A DNA molecule of claim 12 or 13 for use in cancer immunotherapy.

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Fig. 1





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Fig. 2A

FIGURE 2 A)

|                     |   |     |
|---------------------|---|-----|
| CAMEL               | -----   |     |
| LAGE-1 <sup>s</sup> | --ATCCTCGTGGCCCTGACCTTCTCTCTGAGAGCCGGCAGAGGCTCCG    | 48  |
| LAGE-1 <sup>L</sup> | GATCCTCGTGGCCCTGACCTTCTCTCTGAGAGCCGGCAGAGGCTCCG     | 50  |
| NY-ESO-1            | --ATCCTCGTGGCCCTGACCTTCTCTCTGAGAGCCGGCAGAGGCTCCG    | 48  |
| CAMEL               | -----   |     |
| LAGE-1 <sup>s</sup> | GAGCCATGCAGGCCGGAAGCCAGGCCACAGGGGTTTCGACGGGCGATGCT  | 14  |
| LAGE-1 <sup>L</sup> | GAGCCATGCAGGCCGGAAGCCAGGCCACAGGGGTTTCGACGGGCGATGCT  | 98  |
| NY-ESO-1            | GAGCCATGCAGGCCGGAAGCCAGGCCACAGGGGTTTCGACGGGCGATGCT  | 100 |
|                     | *****   | 98  |
| CAMEL               | -----   |     |
| LAGE-1 <sup>s</sup> | GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCCAGGGGCAATGCTGG | 64  |
| LAGE-1 <sup>L</sup> | GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCCAGGGGCAATGCTGG | 148 |
| NY-ESO-1            | GATGGCCCAGGAGGCCCTGGCATTCTCTGATGGCCCCAGGGGCAATGCTGG | 150 |
|                     | *****   | 148 |
| CAMEL               | -----   |     |
| LAGE-1 <sup>s</sup> | CGGCCAGGAGAGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG    | 114 |
| LAGE-1 <sup>L</sup> | CGGCCAGGAGAGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG    | 198 |
| LAGE-1 <sup>L</sup> | CGGCCAGGAGAGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG    | 200 |
| LAGE-1 <sup>L</sup> | CGGCCAGGAGAGCGGGTGCCACGGGCGGCAGAGGTCCCCGGGGCGCAG    | 198 |
|                     | *****   |     |

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Fig. 2A continued

CAMEL  
 LAGE-1<sup>s</sup>  
 LAGE-1<sup>L</sup>  
 NY-ESO-1

164  
 248  
 250  
 248

GGCAGCAAGGCCCTCGGGCCGAGAGGCGCCCCCGGGGTCGCGCAT  
 GGCAGCAAGGCCCTCGGGCCGAGAGGCGCCCCCGGGGTCGCGCAT  
 GGCAGCAAGGCCCTCGGGCCGAGAGGCGCCCCCGGGGTCGCGCAT  
 GGCAGCAAGGCCCTCGGGCCGAGAGGCGCCCCCGGGGTCGCGCAT  
 \*\*\*\*\*

CAMEL  
 LAGE-1<sup>s</sup>  
 LAGE-1<sup>L</sup>  
 NY-ESO-1

214  
 298  
 300  
 298

GCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG  
 GCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG  
 GCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG  
 GCGGTGCCGCTTCTGCGCAGGATGGAAGTGCCCCCTGCGGGGCCAGGAG  
 \*\*\*\*\*

CAMEL  
 LAGE-1<sup>s</sup>  
 LAGE-1<sup>L</sup>  
 NY-ESO-1

264  
 348  
 350  
 348

GCCGGACAGCCGCCCTGCTTCAGTTGCACATCACGATGCCCTTCTCGTCGC  
 GCCGGACAGCCGCCCTGCTTCAGTTGCACATCACGATGCCCTTCTCGTCGC  
 GCCGGACAGCCGCCCTGCTTCAGTTGCACATCACGATGCCCTTCTCGTCGC  
 GCCGGACAGCCGCCCTGCTTCAGTTGCACATCACGATGCCCTTCTCGTCGC  
 \*\*\*\*\*

CAMEL  
 LAGE-1<sup>s</sup>  
 LAGE-1<sup>L</sup>  
 NY-ESO-1

314  
 398  
 400  
 398

CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT  
 CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT  
 CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT  
 CCATGGAAGCGGAGCTGGTCCGAGGATCCTGTCCCGGGATGCCGCACCT  
 \*\*\*\*\*

Fig. 2A continued

|                     |  |     |
|---------------------|--|-----|
| CAMEL               | CTCCCCGACCAAGGGCGGTTCTGAAGGACTTCACCGTGTCGGGCAACCT  | 364 |
| LAGE-1 <sup>s</sup> | CTCCCCGACCAAGGGCGGTTCTGAAGGACTTCACCGTGTCGGGCAACCT  | 448 |
| LAGE-1 <sup>L</sup> | CTCCCCGACCAAGGGCGGTTCTGAAGGACTTCACCGTGTCGGGCAACCT  | 450 |
| NY-ESO-1            | CTTCCCGTGCCAGGGGTGCTTCTGAAGGAGTTCACTGTGTCGGGCAACAT | 448 |
|                     | ** *** .. ***** * ***** ***** ***** *              |     |
| CAMEL               | ACTGTTTAT-----                                     | 373 |
| LAGE-1 <sup>s</sup> | ACTGTTTAT-----                                     | 457 |
| LAGE-1 <sup>L</sup> | ACTGTTTATGTCAGTTCGGGACCAGGACAGGAGGCGCTGGCGGATGA    | 500 |
| NY-ESO-1            | ACTGACTAT-----                                     | 457 |
|                     | ****. ***  |     |
| CAMEL               | -----  | 373 |
| LAGE-1 <sup>s</sup> | -----  | 457 |
| LAGE-1 <sup>L</sup> | GGGTGGTGGGTTGGGGGCTGGGATCCGCCCTCCCCGAGGGCAGAAAGCT  | 550 |
| NY-ESO-1            | -----  | 457 |
| CAMEL               | -----  | 373 |
| LAGE-1 <sup>s</sup> | -----  | 457 |
| LAGE-1 <sup>L</sup> | AGAGATCTCAGAACACCCAAACACAAGGTCTCAGAACAGAGACCTGGTAC | 600 |
| NY-ESO-1            | -----  | 457 |

Fig. 2A continued

|                     |     |   |
|---------------------|-----|---|
| CAMEL               | 373 | -----   |
| LAGE-1 <sup>s</sup> | 457 | -----   |
| LAGE-1 <sup>L</sup> | 650 | ACCAGGCCCGCCGCCAGGAGCCAGGAGATGGGTGCAGAGGTG        |
| NY-ESO-1            | 457 | -----   |
| CAMEL               | 385 | -----CCGACTGACTGC                                 |
| LAGE-1 <sup>s</sup> | 469 | -----CCGACTGACTGC                                 |
| LAGE-1 <sup>L</sup> | 700 | TCGCCTTTAATGTGATGTTCTCTGCCCTCACATTAGCCGACTGACTGC  |
| NY-ESO-1            | 469 | -----CCGACTGACTGC                                 |
|                     |     | *****   |
| CAMEL               | 435 | TGCAGACCAACGCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC |
| LAGE-1 <sup>s</sup> | 519 | TGCAGACCAACGCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC |
| LAGE-1 <sup>L</sup> | 750 | TGCAGACCAACGCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC |
| NY-ESO-1            | 519 | TGCAGACCAACGCAACTGCAGCTCTCCATCAGCTCCTGTCTCCAGCAGC |
|                     |     | *****   |
| CAMEL               | 485 | TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT |
| LAGE-1 <sup>s</sup> | 569 | TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT |
| LAGE-1 <sup>L</sup> | 800 | TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT |
| NY-ESO-1            | 569 | TTTCCCTGTTGATGTGGATCACGCAGTGCTTTCTGCCCGTGTTTTGGCT |
|                     |     | *****   |

Fig. 2A continued

CAMEL  
LAGE-1<sup>s</sup>  
LAGE-1<sup>L</sup>  
NY-ESO-1

CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCAGCCCTGGCGCCCTTCCTA 535  
CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCAGCCCTGGCGCCCTTCCTA 619  
CAGGCTCCCTCAGGGCAGAGCGGCTAAGCCAGCCCTGGCGCCCTTCCTA..850  
CAGCCTCCCTCAGGGCAGAGCGGCTAAGCCAGCCCTGGCGCCCTTCCTA..519  
\*\*\* \*\*\*\*\*

CAMEL  
LAGE-1<sup>s</sup>  
LAGE-1<sup>L</sup>  
NY-ESO-1

GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 585  
GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 669  
GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 900  
GGTCATGCCCTCCTCCCTAGGGAATGGTCCCAGCACGAGTGGCCAGTTCA 669  
\*\*\*\*\*

CAMEL  
LAGE-1<sup>s</sup>  
LAGE-1<sup>L</sup>  
NY-ESO-1

TTGTGGGGCCCTGATTGTTTGTCTGGAGGAGGACGGCTTACATGTTG..635  
TTGTGGGGCCCTGATTGTTTGTCTGGAGGAGGACGGCTTACATGTTG..719  
TTGTGGGGCCCTGATTGTTTGTCTGGAGGAGGACGGCTTACATGTTG..950  
TTGTGGGGCCCTGATTGTTTGTCTGGAGGAGGACGGCTTACATGTTG..719  
\*\*\*\*\*

CAMEL  
LAGE-1<sup>s</sup>  
LAGE-1<sup>L</sup>  
NY-ESO-1

TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA-----679  
TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA-----767  
TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA-----993  
TTTCTGTAGAAAATAAAGCTGAGCTACGAAAAAATAAAAAA-----752  
\*\*\*\*\*

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Fig. 2B

## Protein Translations

## ORF3

LAGE-1<sup>S</sup> MQAEGQGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGAT 40  
 LAGE-1<sup>L</sup> MQAEGQGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGAT 40  
 NY-ESO-1 MQAEGRGTTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGAT 40

LAGE-1<sup>S</sup> GGRGPRGAGAAARASGPRGGAPRGPHGGAASAQDGRCPGCA 80  
 LAGE-1<sup>L</sup> GGRGPRGAGAAARASGPRGGAPRGPHGGAASAQDGRCPGCA 80  
 NY-ESO-1 GGRGPRGAGAAARASGPGGAPRGPHGGAASGLNGCCRCGA 80

LAGE-1<sup>S</sup> RRPDSRLLQLHITMPFSSPMEAEELVRRILSRDAAPLPRPG 120  
 LAGE-1<sup>L</sup> RRPDSRLLQLHITMPFSSPMEAEELVRRILSRDAAPLPRPG 120  
 NY-ESO-1 RGPESRLL**EFY**LAMPF**FAT**PMEAEELARR**SLA**QDAPPLVPVG 120

LAGE-1<sup>S</sup> AVLKDFTVSGNLLFIRLTAADHRQLQLSISSCLQQLSLLM 160  
 LAGE-1<sup>L</sup> AVLKDFTVSGNLL**FMSVRDQDREGAGRM**RVV**GWGLGSASP** 160  
 NY-ESO-1 **VLLKE**FTVSGNLLTIRLTAADHRQLQLSISSCLQQLSLLM 160

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Fig. 2B continued

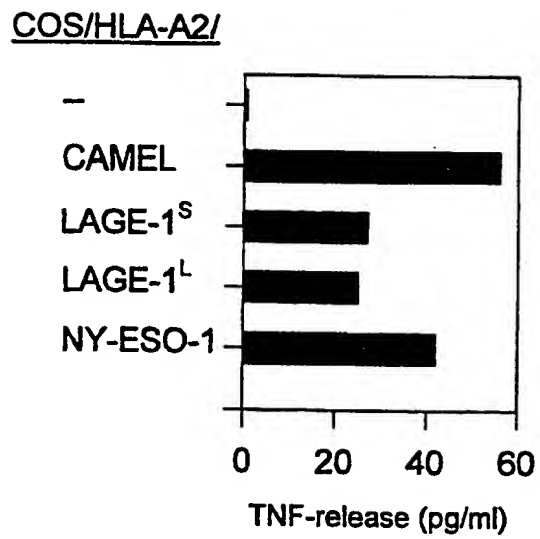
|                     |  |                 |
|---------------------|--|-----------------|
| LAGE-1 <sup>S</sup> | WITQCFLPVFLAQAPSGQRR                                       | 180             |
| LAGE-1 <sup>L</sup> | <b>EGQKARDLRTPKHVSEQRPGTGPPEGAQGDGCRGVA</b>                | 200             |
| NY-ESO-1            | WITQCFLPVFLAQPPSGQRR                                       | 180             |
| LAGE-1 <sup>S</sup> |  |                 |
| LAGE-1 <sup>L</sup> | <b>FNVMFSAPII</b>  | 180 aa, 18.2 kD |
| NY-ESO-1            |  | 210 aa, 21.1 kD |
|                     |  | 180 aa, 18.2 kD |
| <b>ORF1</b>         |  |                 |
| LAGE-1 <sup>S</sup> | MLMAQEALAF <del>MLMAQ</del> GAMLAQERRVPRAAEVPGAQGGQGP      | 40              |
| LAGE-1 <sup>L</sup> | MLMAQEALAF <del>MLMAQ</del> GAMLAQERRVPRAAEVPGAQGGQGP      | 40              |
| NY-ESO-1            | <u>MLMAQEALAF<del>MLMAQ</del>GAMLAQERRVPRAAEVPGAQGGQGP</u> | 40              |
| LAGE-1 <sup>S</sup> | RGREEAPRGVRMAVPLLRMEGAPAGPGGRTAACFSCTSR                    | 80              |
| LAGE-1 <sup>L</sup> | RGREEAPRGVRMAVPLLRMEGAPAGPGGRTAACFSCTSR                    | 80              |
| NY-ESO-1            | RGREEAPRGVRMA <b>ARLQG</b>                                 | 58              |
| LAGE-1 <sup>S</sup> | CLSRRPWKRSWSAGSCPGMPHLSPDQGRF                              | 109 aa, 11.7 kD |
| LAGE-1 <sup>L</sup> | CLSRRPWKRSWSAGSCPGMPHLSPDQGRF                              | 109 aa, 11.7 kD |
| NY-ESO-1            |  | 58 aa, 6.2 kD   |





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Fig. 4

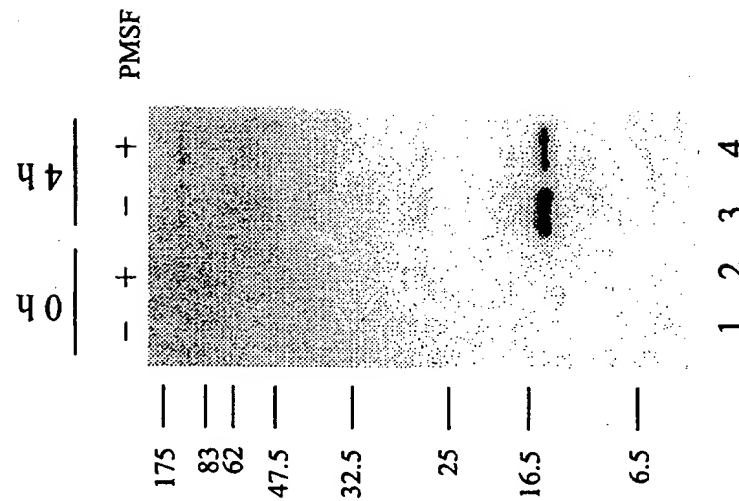


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Fig. 5

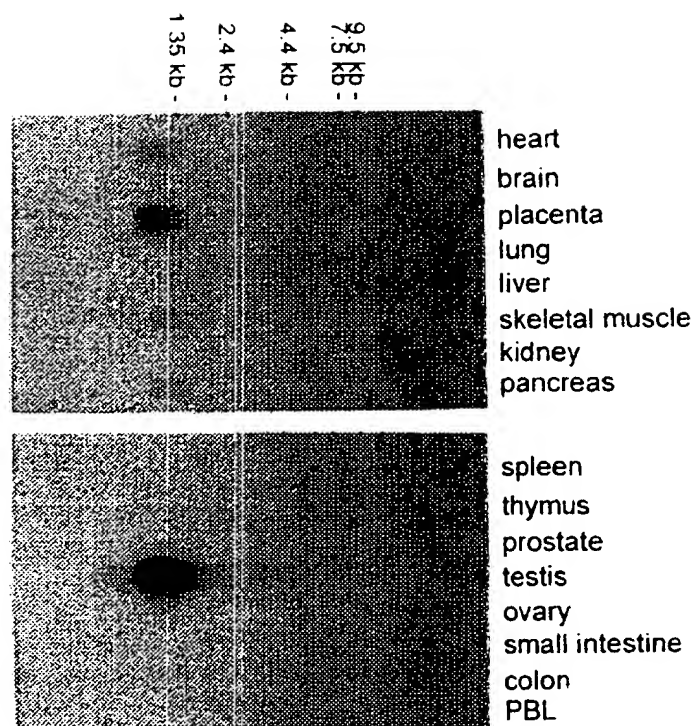


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Fig. 6A



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Fig. 6B

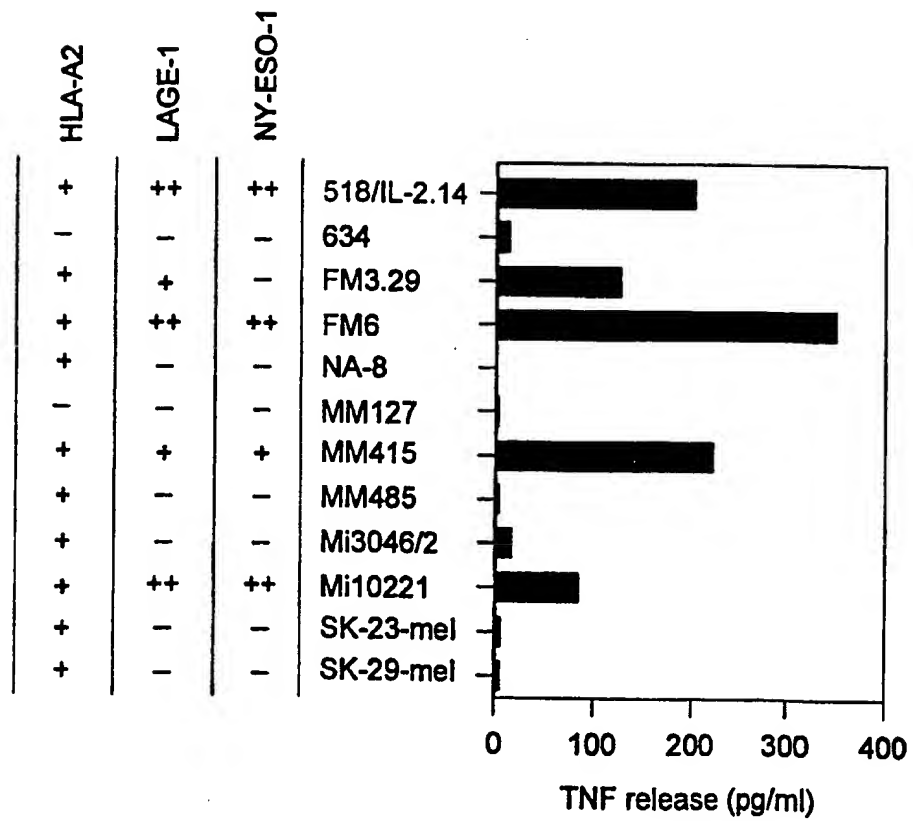
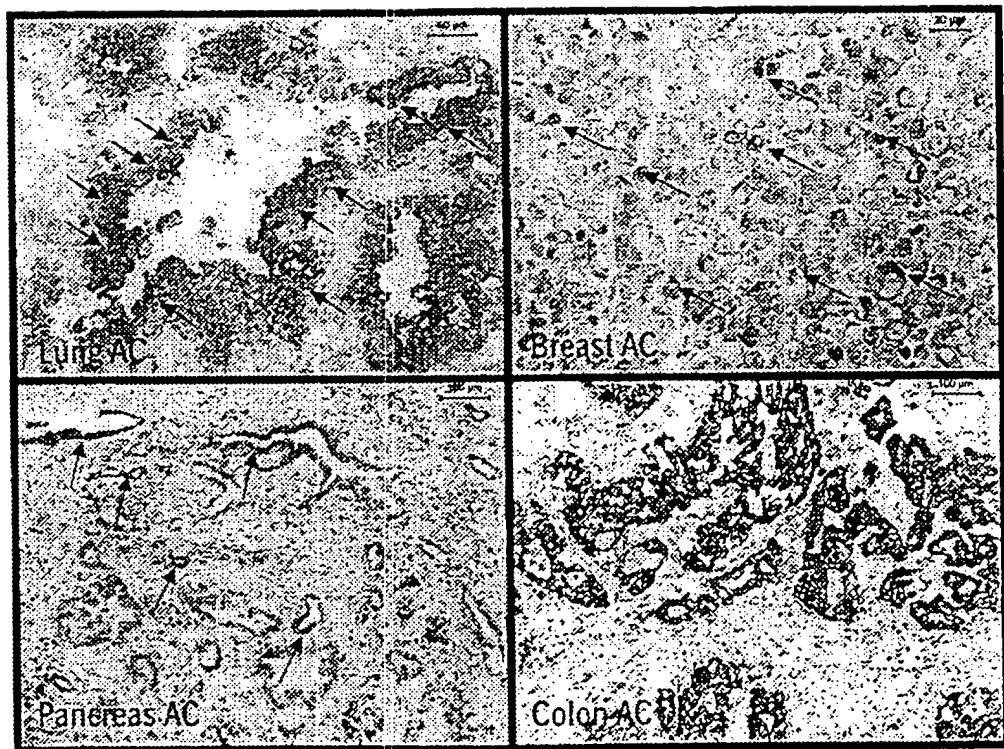


Fig. 7



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Fig. 8

